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(54) Title: FACTORS WHICH INTERACT WITH ONCOPROTEINS

(57) Abstract

The invention relates to polypeptides that bind to the oncogene product mdm2 and the uses of the identified polypeptides in therapeutic compositions to treat aberrant cell division in humans.

54(3) iff entitled to priority

see p 8 & cl. 1 + 2

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App^n: not sarcoma
mouse prostate

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FACTORS WHICH INTERACT WITH ONCOPROTEINS

The invention relates to the oncoprotein mdm2 (murine double minutes), and more specifically, agents which interact therewith and which can thus influence cell growth regulation and which have use particularly, but not exclusively, as therapeutics, diagnostics, prognostics and in assays and as models designed to elucidate cell growth regulation.

The oncoprotein mdm2 (murine double minutes) was originally isolated through its ability to transform mouse BALB/c fibroblast cells (Fakharzadeh, et al., 1991). In the transformed cells, the mdm2 gene is often amplified and exists as a double minute chromosome. The mdm2 gene encodes a protein of 491 amino acids and contains all the domains necessary for being a transcription factor. It has a consensus nuclear translocation signal, two zinc binding domains and acidic and basic domains (Brown, et al., 1993). The mdm2 gene is located on human chromosome 12q13-14 and is often seen to be co-amplified with the CDK4 gene (a cyclin dependent kinase gene which is located within the same region) in human malignant gliomas (He, et al., 1994, Reifenberger, et al., 1995). Amplification of the mdm2 gene has also been found in a variety of human sarcomas (Ollner, et al., 1992; Ladanyl, et al., 1993; Khatib, et al., 1993). Amplification of the mdm2 gene is, however, not universal; many tumours, including some types of leukaemia, were found to have no amplification of the mdm2 gene (Ridge, et al., 1994). Nevertheless, abnormal expression of the mdm2 gene has been found in many types of human tumour. Abnormal expression of mdm2 has been reported in chronic lymphocytic leukaemia (Watanbe, et al., 1994; Huang, et al., 1994). Elevated mdm2

expression was also found in Hodgkin's and non-Hodgkin's lymphomas at both mRNA and protein levels (Chllosl, et al., 1994, Finnegan, et al., 1994). High levels of expression of the mdm2 gene have been linked to a poor response to chemotherapy and short survival in haematological malignancies (Quesnel, et al., 1994).

The best known function of mdm2 is its ability to bind to the tumour suppressor protein p53 via its transcriptional activation domain (Lin, et al., 1994), thus inhibiting the p53 transcription activity (Momand ct al., 1992). In addition, mdm2 has been shown to be able to block p53 induced apoptosis in some cell lines. A recent study also demonstrated that inactivation of the mdm2 gene can result in the embryonic lethality, a phenotype that could be rescued by a p53 null background. All these observations suggest that one of the important functions of mdm2 is its ability to block the activity of p53. However, recent reports showed that mdm2 can also bind to another tumour suppressor gene product Rb (Xiao, et al., 1995) as well as a cell cycle transcription factor E2F1 (Martin, et al., 1995). Interestingly, all three tumour suppressor proteins, p53, Rb and E2F1 (Field et al, 1996), are key players in controlling cell cycle progression and apoptosis, suggesting that mdm2 may play a key role in regulating cell growth. Although the mdm2 protein has many features characteristic of transcription factors and the phenomena listed above are well established, there is little understanding of the true biological function of mdm2. It is, however, clear that mdm2 occupies a key role in cell growth regulation.

A biological role for mdm2 is suggested by the following data. Using FACS analysis as described in Hseih et al 1997, cells selected containing a sub-G1 DNA content (typical of cells undergoing apoptosis) it has been shown that in

the presence of Rb the anti-apoptotic function of mdm2 is abolished. It is known that mdm2 targets p53 for degradation, possibly via the ubiquitination pathway. In the presence of Rb, p53 is stabilised. Therefore interaction between Rb and mdm2 can promote the tumour suppressor activity of p53.

Mdm2 interacts with and is phosphorylated by cyclinA-cdk2 (a S-phase cyclin – cdk complex Lu unpublished data). It has been shown previously that cyclins are targets for ubiquitination. As p53 is also a target for ubiquitination it is possible that mdm2 interaction is causally related to degradation of these important cell-cycle proteins or indeed to the ubiquitination of mdm2. Saos-2 and HI299 cells (null for p53) treated with the proteosome inhibitor ALLN (a calpain proteinase inhibitor) results in an increase in the cellular levels of mdm2. This suggests that mdm2 is a target for ubiquitination in the absence of p53. It may therefore be possible to regulate the negative effects of mdm2 on p53 by alternative strategies.

It is therefore an object of the invention to identify agents which interact with mdm2 and thus have therapeutic, diagnostic or prognostic application.

It is yet a further object of the invention to identify agents which can influence cell growth and regulation via binding to mdm2.

It is yet a further object of the invention to elucidate the role of mdm2 in cell growth regulation by identifying agents that interact therewith with a view to then determining the nature of the pathway involved and thus agents which may be of potential use in regulating said pathway.

It is yet a further object of the invention to provide a methodology for identifying the aforementioned agents.

It will be apparent that agents which interact with mdm2 i.e. those sequences identified in Tables 1 and 2 have potential as therapeutic agents in various processes relating to cell growth and division. A relatively recent alternative strategy to traditional therapies is the use of antisense molecules to regulate the production and/or availability of translatable mRNA to targeted nucleic acid sequences. Clearly some of the identified sequences show elevated expression in certain transformed cells and their down regulation has been linked to a reversion of the transformed phenotype.

As mentioned above, the invention embraces antisense oligonucleotides that selectively bind to a nucleic acid molecule presented in Tables 1 and 2, to decrease transcription and/or translation of these genes. This is desirable in virtually any medical condition wherein a reduction in gene product expression is desirable, including to reduce any aspect of a tumour cell phenotype attributable to the expression of that sequence. Antisense molecules, in this manner, can be used to slow down or arrest such aspects of a tumour cell phenotype.

As used herein, the term "antisense oligonucleotide" or "antisense" describes an oligonucleotide that is an oligoribonucleotide, oligodeoxyribonucleotide, modified oligoribonucleotide, or modified oligodeoxyribonucleotide which hybridizes under physiological conditions to DNA comprising a particular gene or to an mRNA transcript of that gene and thereby, inhibits the transcription of that gene and/or the translation of that mRNA. The antisense molecules are

designed so as to interfere with transcription or translation of a target gene upon hybridization with the target gene. Those skilled in the art will recognise that the exact length of the antisense oligonucleotide and its degree of complementarity with its target will depend upon the specific target selected, including the sequence of the target and the particular bases which comprise that sequence. It is preferred that the antisense oligonucleotide be constructed and arranged so as to bind selectively with the target under physiological conditions, i.e., to hybridize substantially more to the target sequence than to any other sequence in the target cell under physiological conditions. Based upon the DNA sequence presented in Tables 1 and 2 or upon allelic or homologous genomic and/or DNA sequences, one of skill in the art can easily choose and synthesize any of a number of appropriate antisense molecules for use in accordance with the present invention. In order to be sufficiently selective and potent for inhibition, such antisense oligonucleotides should comprise at least 7 (Wagner et al., *Nature Biotechnology* 14:840-844, 1996) and more preferably, at least 15 consecutive bases which are complementary to the target. Most preferably, the antisense oligonucleotides comprise a complementary sequence of 20-30 bases. Although oligonucelotides may be chosen which are antisense to any region of the gene or mRNA transcripts, in preferred embodiments the antisense oligonucleotides correspond to N-terminal or 5' upstream sites such as translation initiation, transcription initiation or promoter sites. In addition, 3'-untranslated regions may be targeted. Targeting to mRNA splicing sites has also been used in the art but may be less preferred if alternative mRNA splicing occurs. In addition, the antisense is targeted, preferably, to sites in which mRNA secondary structure is not expected (see, e.g., Sainio et al., *Cell Mol. Neurobiol.* 14(5):439-457. 1994) and at which proteins are not expected to bind. Finally, although Tables 1 and 2 discloses

cDNA sequence, one of ordinary skill in the art may easily derive the genomic DNA corresponding to the cDNA of Tables 1 and 2. Thus, the present invention also provides for antisense oligonucleotides which are complementary to the genomic DNA corresponding to Tables 1 and 2. Similarly, antisense to allelic or homologous DNAs and genomic DNAs are enabled without undue experimentation.

In one set of embodiments, the antisense oligonucleotides of the invention may be composed of "natural" deoxyribonucleotides, ribonucleotides, or any combination thereof. That is, the 5' end of one native nucleotide and the 3' end of another native nucleotide may be covalently linked, as in natural systems, via a phosphodiester internucleoside linkage. These oligonucleotides may be prepared by art recognised methods which may be carried out manually or by an automated synthesizer. They also may be produced recombinantly by vectors.

In preferred embodiments, however, the antisense oligonucleotides of the invention also may include "modified" oligonucleotides. That is, the oligonucleotides may be modified in a number of ways which do not prevent them from hybridizing to their target but which enhance their stability or targeting or which otherwise enhance their therapeutic effectiveness.

The term "modified oligonucleotide" as used herein describes an oligonucleotide in which (1) at least two of its nucleotides are covalently linked via a synthetic internucleoside linkage (i.e., a linkage other than a phosphodiester linkage between the 5' end of one nucleotide and the 3' end of another nucleotide) and/or (2) a chemical group not normally associated with

nucleic acids has been covalently attached to the oligonucleotide. Preferred synthetic internucleoside linkages are phosphorothioates, alkylphosphonates, phosphorodithioates, phosphate esters, alkylphosphonothioates, phosphoramidates, carbamates, phosphate triesters, acetamidates, peptides, and carboxymethyl esters.

The term "modified oligonucleotide" also encompasses oligonucleotides with a covalently modified base and/or sugar. For example, modified oligonucleotides include oligonucleotides having backbone sugars which are covalently attached to low molecular weight organic groups other than a hydroxyl group at the 3' position and other than a phosphate group at the 5' position. Thus modified oligonucleotides may include a 2'-0-alkylated ribose group. In addition, modified oligonucleotides may include sugars such as arabinose instead of ribose. Modified oligonucleotides also can include base analogs such as C-5 propyne modified bases (Wagner et al., *Nature Biotechnology* 14:840-844, 1996). The present invention, thus, contemplates pharmaceutical preparations containing modified antisense molecules that are complementary to and hybridizable with, under physiological conditions, nucleic acids presented in Tables 1 and 2, together with pharmaceutically acceptable carriers.

Antisense oligonucleotides may be administered as part of a pharmaceutical composition. Such a pharmaceutical composition may include the antisense oligonucleotides in combination with any standard physiologically and/or pharmaceutically acceptable carriers which are known in the art. The compositions should be sterile and contain a therapeutically effective amount of the antisense oligonucleotides in a unit of weight or volume suitable for

administration to a patient. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredients. The term "physiologically acceptable" refers to a non-toxic material that is compatible with a biological system such as a cell, cell culture, tissue, or organism. The characteristics of the carrier will depend on the route of administration. Physiologically and pharmaceutically acceptable carriers include diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials which are well known in the art.

According to a first aspect of the invention there is therefore provided the use of at least one agent as identified in Table 1 or 2, or a homologue or analogue thereof, or a functionally effective fragment thereof, for binding with mdm2 and thus influencing cell growth regulation.

It will be apparent that the term agent can refer to any small molecule or ligand (e.g. antibody) that can bind to mdm2 and thus interfere with mdm2 function. Since, it is known that interactions of mdm2 with the tumour suppressor proteins p53 and Rb imply mdm2 prevents tumour suppressor function it therefore follows that those agents that interfere with the interaction between mdm2 and p53 or Rb will release p53 or Rb from mdm2 and so stop cell growth. These agents therefore can significantly influence the functioning of mdm2 and moreover will have use as therapeutics.

According to a further aspect of the invention there is provided a therapeutic composition comprising at least one agent as identified in Table 1 or 2, or a homologue or analogue thereof, or a functionally effective fragment thereof, and a suitable excipient or carrier.

According to a yet further aspect of the invention there is provided a diagnostic or prognostic assay for predicting or determining cell growth regulation which assay comprises identifying the existence of, or cellular levels of, any one or more of the agents identified in Table 1 or 2, or a functionally effective fragment thereof, with a view to determining the likelihood of, or existence of, mdm2 binding to said agent or said fragment.

It will therefore be apparent that given that mdm2 interacts with the agents identified in Table 1 or 2 then the degree of interaction will have an effect on cell growth regulation given the role that mdm2 has to play in cell growth regulation. Therefore, an assay to determine the nature of this interaction will have use in determining aspects of cell growth regulation.

In a preferred embodiment of the invention the assay may be a competitive binding assay of a conventional nature such as a radio-immunoassay or an immunoassay. Assays of this nature are well known to those skilled in the art and so need not be described herein in detail.

According to a further aspect of the invention there is provided a methodology for identifying agents that interact with mdm2 which methodology employs a yeast two hybrid assay as herein described.

Notably, insofar as the agents identified in Table 1 or 2 are concerned OS-9 is amplified in human sarcomas and furthermore over expression of S3 and C140 is found in human colon rectal and thyroid tumours respectively. This tends to imply that not only do these agents have a role to play in influencing the

functioning of mdm2, as aforedescribed, but conversely, mdm2 may have a role to play in effecting the function of a OS9, S3 and C140. Thus, it follows, that in some instances mdm2 may have a role to play as a therapeutic agent in treating at least human sarcomas, human colon rectal tumours and human thyroid tumours.

Moreover, it is also important to note that mdm2 interacts with human DNA binding protein (Enhancer Factor 1) which can bind to erb-B-2 and EGFR. It therefore follows that mdm2 may control erb-B-2 expression in human breast tumours and thus, again, agents which can interfere with the activity of mdm2 may be used to prevent its binding with human DNA binding protein and so prevent expression of erb-B-2.

Further evidence for the involvement of mdm2 in breast cancer is given by the following data. If an mdm2 expression plasmid and a reporter plasmid containing the multi-drug resistance gene mdr1 are co-transfected into human breast carcinoma cell line MCF-7, a 50-200 fold inhibition of transcriptional activity of the mdr1 gene is detected. The mdr1 gene is regulated at the level of transcription and the promoter of mdr1 has been shown to bind the transcription factor YB-1, a protein herein described as a target for mdm2.

Further, it is known that mdm2 interacts with L6/TAXREB107 which in turn binds to Tax responsive element in the LTR region of HTLV-1. It therefore follows that mdm2 can effect the function of TAX and the activity of HTLV-1. It is also possible that it may effect the activity of HIV since there are sequence similarities between HTLV-1 and HIV. It therefore follows that agents that bind with mdm2 may prevent the interaction of same with L6/TAXREB107 and

so may be used to alleviate or treat symptoms associated with expression of HTLV-1 or HIV genes.

Further, mdm2 interacts with S3, S7, L6, EF1α and YB-1. Since these are all important proteins involved in protein translation it follows that mdm2 has a key role to play in controlling protein synthesis. Accordingly, any one or more of the agents listed in Table 1 or 2, other than S3, S7, L6, EF1a and YB-1 may be used to inhibit protein synthesis.

In addition, mdm2 interacts with ERCC3/XP-B which is a key component in nucleotide excision repair, for example, to repair UV damaged DNA. Indeed, the human homologue of ERCC3 ie XP-B is the gene lacking in the Group B Patients suffering from cancer prone syndrome xeroderm pigmentosom. ERCC3XP-B is also a component of transcriptional factor TFIIH. ERCC3/XP-B is also implicated in p53 induced apoptosis and this may be mediated by its ability to interact with p53. The ability of mdm2 to interact with ERCC3/XP-B may represent another means by which mdm2 can influence p53 expression and thereby control DNA repair and apoptosis. It therefore follows that ERCC3/XP-B is important in gene expression. Accordingly, any one or more of the agents listed in Table 1 or 2, other than ERCC3/XP-B, may be used to interfere with gene expression.

According to a further aspect of the invention there is provided use of an agent identified in Table 1 or 2, other than S3, L6 or EF1a, or a homologue or analogue thereof, or functionally effective fragment thereof, to inhibit protein synthesis.

Further, it is of note that S3 also comprises AP endonuclease activity as well as endonuclease III activity. Therefore mdm2 would seem to have a role to play in DNA repair and accordingly agents other than S3 or AP listed in Table 1 or 2 may be used to inhibit said repair and so affect damaged DNA processing.

According to a yet further aspect of the invention there is provided use of an agent identified in Table 1 or 2, other than S3 or AP, or a homologue or analogue thereof, or functionally effective fragment thereof - to inhibit DNA repair.

Further, since mdm2 has oncogenic activity its activity may be modulated by its interaction with any one or more of the proteins listed in Table 1 or 2.

Embodiments of the invention will now be described, by way of example only, with reference to the following methodology and Table 1 and 2 wherein;

Table 1 is a list of known agents which have been shown to bind to mdm2 using the methodology hereinafter described and Table 2 represents the corresponding DNA sequence structure of the agents listed in Table 1 and also the DNA sequence structure of other agents whose sequence structure has been elucidated but whose characterisation is unknown.

Isolation of Novel mdm2 Interacting Proteins

The yeast two hybrid assay is based on the fact that many eukaryotic transcriptional activators (GAL4 for example) consist of two physically separable modular domains, such as DNA binding and transactivation domains.

When two separated domains such as DNA binding and transcriptional activation domains are brought together through protein-protein interactions, they can function as a transcription activator and turn on the relevant reporter genes. Yeast strain HF7c contains two different GAL4 reporter genes HIS3 and LacZ under the control of dissimilar promoters. Using this yeast two hybrid system, Human mdm2 cDNA was cloned into a yeast expression vector pGBT9 to produce a GAL4 binding domain containing mdm2 fusion protein. The pGBT9-mdm2 plasmid was then transformed into yeast strain (HF7c). pGBT9-mdm2 alone is not sufficient to turn on the reporter gene in yeast HF7c. pGBT9-mdm2 transformed yeast colonies were expanded and their mdm2 expression was detected by anti-mdm2 antibody SMP14. Clones expressing mdm2 at high level were grown into large quantities and used to carry out the library screening. A human B cell cDNA library (Gift from Dr. Steve Elledge) cloned into yeast expression vector pACT, which contains the transcriptional activation domain of GAL4, was subsequently transformed into pGBT9-mdm2 containing HF7c strain. The library screening and selection procedure were mainly based on the manual from Clontech (matchmaker™ two hybrid system). If mdm2 interacts with a protein derived from the yeast expression library, such interaction will bring together the GAL4 DNA binding domain (in pGBT9-mem2) and transactivation domain (from pACT vector which was used to construct the library). Such interaction will then transactivate the expression of HIS3 and LacZ genes. The expression of HIS3 will allow the yeast to grow in the medium lacking the amino acid histidine. Transactivation of LacZ gene will turn on the β-galactosidase activity and this can make the transformed yeast colonies turn blue in a β-galactosidase assay.

The transformed yeast were selected for their ability to grow in the medium

lacking the amino acid Histidine. The resistant colonies were then tested for their β-galactosidase activity. The colonies which turned blue within 2 hours in the β-galactosidase assay were isolated and cultures on 3-AT(3-aminotriazole) containing plate in order to select the strong interacting proteins. The resistant colonies were cultured and the DNA from these colonies were isolated. The DNAs were analysed by PCR to identify the known interacting proteins such as p53 and Rb. The DNAs other than p53 and Rb were then transformed into E.Coli HB101 under Leu selection to eliminate the pGBT9 plasmid. The DNA from HB101 was then subsequently transformed into E.Coli DH5a. DNAs from these DH5a colonies were then isolated and re-transformed into yeast strain HF7c with either pGBT9-mdm2 (positive control) or pLAM5[1] (negative control). The DNAs which can result in the yeast growth in the absence of amino acid histidine and turn on the β-galactosidase activity when co-transformed with pGBT9-mdm2 but not pLAM5[1] were considered as true positives. In total, 26 positive clones were identified and among them 4 contain the same sequence.

Implications of mdm2 Function Through its Interaction with the Identified Proteins

Using the sequence from the identified clones to search the gene data base, it was clear that the sequences derived from four of the clones were encoding for four different unknown proteins as summarised in Table 1 or 2. Among the characterised sequences, the sequence identity to known genes is summarised in Table 1.

It can therefore be seen that we provide an assay for identifying agents that bind

to mdm2 and so have a role to play in many aspects of cell processing.

Table 1

Yeast clones	names of the known genes and functions
SZ7	Elongation factor 1a protein translation
SZ10	Human DNA binding protein (1) DNA binding protein, transcription enhancer (2) Binds to the promoters of erb-B-2 and EGFR
SZ15	Cytochrome C
SZ16	OS-9 (1) ubiquitously expressed (2) amplified in human sarcomas
SZ17	Sequence from yeast two hybrid assay
SZ18	Ribosomal protein S3/v-fos transformation effector Fte-1 (1) Component of ribosomal small subunit, binds to 18S RNA. (2) Recombinant S3 and purified S3 from mouse cells contain AP1 endonuclease activity as well as endonuclease III activity which are important in DNA repair. (3) Overexpression in Fanconi's anemia cell lines will cause resistance to DNA cross linking agents such as mitoycinC and dlepoxybutane. (4) E.coli S3 is a DNA binding protein. (5) S3 locates in nucleus and cytoplasm (6) Fte-1 is overexpressed 4-5 fold in v-fos transformed cells. Fte-1 level reduced to normal level in v-fos revertant cells. (7) Overexpressed in human colon cancers and polyps.
SZ27	cytochrome oxidase I.II

SZ38	Ribosomal protein L6/TAXREB107. C140 (1) L6 is a component of ribosomal large subunit and it can bind to 5sRNA. (2) TAXREB107 is a DNA binding protein and it binds to HTLV-1 tax responsive element. (3) L6/TAXREB107 has very high sequence homology to C140. C140 is markedly increased in malignant transformed thyroid tumour cells, 5.8 fold higher than the normal.
SZ12	Ribosomal protein S7
SZ34	ERCC3/XP-B (1) Human homolog of ERCC3 is XP-B,which is the gene lacking in the group B patients suffering from cancer prone xeroderma pigmentosom. (2) ERCC3/XP-B is a key component in nucleotide excision repair (to repair UV damaged DNA). (3) ERCC3/XP-B is also a component of transcription factor TFIIH, therefore it involves in general transcription.

Table 2

SZ7/ elongation factor 1a

GCCACGAAGGCCAACTCGTCCAACTGACAAGCCCTTNCNCCTGCCTCTCCA
GGATGTCTACAAAATTGGTGGTATTGGTACTGTTCCTGTTGGCCGAGTGG
AGACTGGTGTTCTCAAACCCGGTATGGTGGTCACTTTGCTCCAGTCAACGT
TACAACGGAAGTAAAATCTGTCGAATGACATGAGCTTTGAGTGAAGCTCTTCCTG
GGACATGTGG

SZ10/dbp
CCGCTCCCGAAGCTGANCAGGGCGGGGCTGANTNAATGCCGGCTTACCATCTCTA
CCATCATCCGGTTTAGTCATCCAACAAGAAGAAATATGAAATTCCNGCCNTNNGA
AATGAACNAAAGATTGGAGCTGAAGACCTAAANTGCTTGCTTTTTTGGCCCGTTT
GACCCNATTAATTTGAACTTTCTGCCTTTATCTNNTTCCNCCNTGGGGGTTTTTTTT
TATTTTTTTACCCTAAAANAACTTTCTCCTTTTTTGGGTTAATTAAACCAAAACCTT
TTTTTTTTTTTAANAAAAAAACCCCTGGGTTTTTTTTCCTCCAATTTAACCNCCCCT
TTTTAAAANGGGTTTTTTTTTAAAAATTTGGNTTTTCCCATTANTCCTGGGGTTCC
CCANTTTTTGNAAAAAATTTTTTTTNAAAGAAAAACTTTTCCNNTTTTTTTTTNAA
ATTTTTTGGNTTNNATTNAAAAAN

SZ15/cytochrome C
GCCACGAAGGCATGTTGAGAAAGGCAAGAAGATTTTTATTATGAAGTGTTCCCAG
TGCCACACCGTTCAAAAGGGAGGCAAGCACAAGACTGGGCCAAATCTCCATGGT
CTCTTTGGGCGGAAGACAGGTCAGGCCCCTGGATTACTCTTACACAGCNNCAATA
AGAACAAAGGCATCATCTGGGAGAGGATACACTG

SZ16/OS-9
GCCACTAAGGCGCCACGAAGGCTCAGACCGAGACCGGCTCCGTTCGGAGACAGA
GAAAGAGCTGGACCCAGATGGGCTGAAGAAGGAGTCAGAGCGGGATCGGGCAA
TGCTGGCTCTCACATTCCACTCTCAACAAACTCATCAAAAGACTGGAGGAAAAAG
AGAGTCAGATGCTGGTGAAG

SZ17/Hela sequence
GCCACGAAGGCGTGAACGAAGCGGTGGGGAGCAGGCACAGGACTGGGATGCTCT
GCCACCCAAGCNGCCCCGACTACGAGGGAAACAAGATCGGAGNCCGTACNTATT
GTGGTGCTGGAAGGGTCCAGTCTGGAGACAGTCAAGGTAGGGAAGACATATGCT
ACTCAACTGTGACAGCACAAGTCTATATTGTTGAAGAAT

SZ18/S3/Fte-1
GCCACGAAGGCCGCTCGTCACCAGGACCCAAGGAACCAAAATTGCATCTGATGG
TCTCAAGGGTCGTGTGTTTGAAGTGAGTCTTGCTGATTTGCAGAATGATGAAGTT
GCATTTAGAAAATTCAAGCTGATTACTGAAGATGTTCAGGGTAAAANCTGCCTGA
CTAACTTCCATGGCATGGATCTACCCGTGACAAATGTGTTCCATGGCTAAAA

SZ27/cytochrome oxidase I. II

GCCACGAAGGCGTAAAACCGACCCCATGACCCCTAACAGGGCCCTCTCAGCCCTC
CTAATGACCTCCGGCCTAGCCATGTGATTTCACTTNCACTCCATAACGCTCCTCAT
ACTAGGCTACTAACCAACACACTAACCATATACCAATGATGGCCGCATGTAACAC
G

SZ38/L6/TAXREB107, C140
GCCACGAAGGCCCGCAACCCTGTCCTTGTCAGAGGAATTGGCAGGTATTCCCGAT
CTGCCATGTATCCAGAAAGGCCATGTACAAGAGGAAGTACTCAGCCGCTAAATCC
AAGGTGAAAAGAAAAAGAAGGAGAAGGTTCTCGCAACTGTTACAAAACCAGTTG
GTGGTGACAAGAACTGGCACTGGATCCCGTGTGGTTAACTTCGCAAA

SZ12/S7
GCCACGAAGGCAGAAAATCCAAGTCCGGCTAGTACGCGAATTGGAGAAAAAGTT
CAGTGGGAAGCATGTCGTCTTTATCGCTCAGAGGAGAATTCTGCCTAAGCCAACT
CGAAAAAGCCGTACAAAAAATAAGCAAAAGCGTCCAGGAGCCGTACCTGACAGC
TGTGCACGA

SZ34/ERCC3/XP-B
GCCACGAAGGCAGGAATATGCCATTCGACTGAACAAACCCTATATCTACGGACCT
ACGTGTCAGGGGGAAAGGATGCAAATTCTCCAGAATTTCAAGCACAACCCCAAA
ATTAACACCATCTTCATATCCAAGGTAGGTGACACTTCGTTTGATCTGCCGGAAG
CAAATTGTCTCATTCAGATCTCATCCCATGGTGGCTCCAGGCGTCAGGAAGCCAA

UNKNOWN SEQUENCES
SZ29
CACTACAATGGATGATGTATATAACTATCTATTCGATGATGAAAGATACCCCACC
AAACCCAAAAAAAGAGATCTGGAATTCGGATCCTCGAGGCCACGAAGGCCGGAA
ATCTGAAGCAAAGAAGGAATCACTTCCCAGAAGAAGAAGCCTGCATATCGAACC
TTTATTAAAGGAAAAAATTACATGAAGCAACGGGATGACATTTTGATTAACAGGC
CNGCAAAGAANCACCTANAATTGTATGACAGGGATCTGAAACATTTTCGGATCTC
TAAGGCNCTCGATANANTTCTTGATCCCACTTGTNCAATAAAGACACCCGAGATT
NCGGTGTCCATCATAAAGGAGTTNAATCGAAGAAGAGTCCTTGCAAATGCGCTTG
CANGTCTGGATGAAAAAGAAATCANTCNTGTTCTTAATTTTTTGATAANGAATCT
NTCTCCCCNAGATTTGCCCCTGTTTTAATCNNTGCTGCTGAAATAATTATTGATAT
ATATCTGCCTGTNATTGGTCCTCCCCTGTANTTGATAAAAAGTTTTTACTACTTCN
NGGACTTGTTTAAAAAGANATTGATTNCCCAGANAATTGTTNAAACCTTGGGGGA
TGATNGATATGCTNTTGCCCCNTGAAAAAGAAGGAAGGCCNTCTTTNTTTGNACA
CCCCTCTGATGGATTCCCCAAAATAANAAAATAAATCCTNNTGTCTGCTAATAAA
ACTNTAAAACTCCNAAATTTGAATAAATTTGACTGTTTTNATTTTTGGGAAAAAA
NCNCTTTGATACTTTTAAAAACTGTTTGCNAAACCCTTCTNTGGAAAAAACGGAA
TAATTTTGGCCGGAAACCATTTCCCTTTTAANTTTAAAATGGTTTCCCCTTTTNTTT
TGAATTTTACCCCTCCCCNNAAAATTTCCNTTTTTTTTCCCCAATTTGTTTTCNTGG
GATGGAATTTANTTTTATACCGGATTTTTTTTTTCCCCNTTTGGTTTGTTTTCCATT
GGGAATCCATTTAAACCCCCCNGGAAAAGGTCCCTNGGAACCN

SZ2/unknown
GCCACGAAGGCGTTTCACGTCTTCGCCAATCACAGTGCAGCAAGGCCAATTCTCT
CAGAAACCCCCACGTGTGCACAGTGGGAGAGGGGAAAGAGAAAAAAAGGTGAG
CATGGAGGAAAAAGGTACTGGATAAAGTAAACTTCAAACCTTAGGGC

SZ33
GNNNNNNNNTTNCTCCNCTACAATGGATGATGTATATAACTACCTATTCGATGAT
GAAGATACCCACCAAACCCAAAAAAAGAGATCTGGAATTCGGATCCTCGAGGCC
ACGAANGCCGGCCTTCGTGGCCGAAAAGAGCTGAGCGGAGACCAAAGTCAGCCG
GGAGACAGTGGGTCTGTGAGAGACCGAATAGAGGGGCTGGGGCCACGAGCGCCA
TTGACAAGCAATGGGGAAGAAACAGAAAAACAAGAGCGAAGACAGCACCAAGG
ATGACATTGATCTTGATGCCTTGGCTGCAGAAATAGAANGAGCTGGTGCTGCCAA
AGAACAGGAGCCTCNAAAGTCNAAAGGGAANAAGAANAAAGAGAANANNAAGC
AGGACTTTGATGAAGATGATATCCTGAAAGAACTGGAAGAATTGTCTTTGGAAGC
TCAAGGCATCNAAGCTGACAGANAAACTGTTGCAGTGAAGCCNACAGAAAACAA
TGAAGAAGAATTCCCCTCCNAGATNNNNNNNAGAAAGGACCGAAGGGCNAAAA
ACAGAATTTTGATGATAATGATACGAAGAATTGGAAGATAAAGATTCCNAATCC
ANAAAGACTGCNAAACCGAAAGTGGAAATTTCTCTGGGANTGATGATGATGATG
ATTTTAACCAACTTCCTAAAAAACTNAAGGGAAAGCTCCNAAATCAATAANAAA
TTGGATGGTCCAAGAAGATGAGGATACCTTNNNAATTTANGANCTTCANAATAAT
TCTCTGGTGAAATTGTGATAATCCATAATTTTGCNTCTTNAAANGGCGAAAAAN

TCNAAAACCNCCCGTTCTNCCTTNAANTTGGATGAAAATATACCCNCCTCCAATT
AAACNTGGCCCNAANAAGGCAANNNAGGACNCAANAAAAAACCAAATAAAAAA
ACNAACNCGGAACTGAANAAAAAAAAATTTAAACCGTTTNGGTTTAATTTCCNN
GGTTNCCCGGGANTTNAAAAAAAANTTGCCTCCTGCCCCAAATTNTTAANCN

SZ37
GNNNNGNNTTCNNCCACTACAATGGGATGATGTATATAACTACCTATTCGATGAT
GAAGATACCCACCACACCCNAAAAAAAGAANNTCTNGGATTCCGGNTCCTCGAG
GCCACGAAAGGCCGGCCTTCGTGGCCGAAAAGAGCTGAGCGGAGACCAAAGTCN
GCCGGGAGACAGTGGGTCTGTGAGAGACCGAATAGAGGGGCTGGGGCCACGAGC
GCCATTGACAAGCAATGGGGAAGAAACAGAAAAACAAGAGCGAAGACAGCACC
AAGGATGACATTGATCTTGATGCCTTGGCTGCANAAATAGAAAGAGCTGGTGCTG
CCNAAGAACAGGAGCCTCNNAAGTCTCNAGGGAAANAGAAAANAGAGAATANN
NAGCCCGACTTTGATGAAGATGATATCCTGAAAGAACTGGAAGAATTGTCTTTGG
AACTCNNGGCATCTAAGCTGACAGANAAACTGTTGCNNTGAAGCCCCCANAAAA
CNATGAAGAAGAATCCCCTCCCNAGATTNNNNANAGAAAGGACANANGGGCCN
NAACAGANTTTTGATGATAATGATAGCGAANAATTGGAAGATATAGATTCNCAA
TCNCNNNAGACTGCCCANCCNAAAGTGGAAATTTNTCTNGGAGTGATGATGATG
ATGATTTAACANACTTCCTCNAAAANCTNNAGGGAAAGCTCCAAATCNNATAAN
AAATNNGATGGGTCTCAAGAAGATNAGGATACNTTTNNNATTTTNAACGTCCCNA
ATAAATTCCTCTNGTGAAATTGTGATAATCCATAATTTTTGCCTCTCCAAAAGGAC
CAAAAAAATCCAAAANCCNCCCCNTCCCCCTTNAAATTNGATTANAATTTACCCN
CCTCCCATTTAAACCTGNCCCNNAAGGCGAAAAAAAGGGCNCCNANNAAANNCC
CANTAATAANAACCNACCGCGGGGCTTANNAAANAAAAATTTAACCGTTTTNGG
TTNATTTCCNCNGGACCCCCNGGATTTTAAAAAACTTTGCCTCCTGGCCCCAAATT
TTTAATCTAA

CLAIMS

1. The use of an agent represented in Table 1 or 2, or a homologue or analogue thereof, or a functionally effective fragment thereof, for binding with mdm2 and thus modulating cell division regulation.

2. A therapeutic composition comprising at least one of the agent represented in Table 1 or 2, or a homologue or analogue thereof, or a functionally effective fragment thereof, for use in influencing mdm2 activity and also including a suitable carrier dilutant or excipient.

3. A diagnostic/prognostic assay kit for determining cell division regulation comprising means for determining the existence and/or cellular levels of at least one polypeptide represented in Table 1 or 2, or an active fragment thereof, with a view to determining the presence of mdm2 binding to said polypeptide.

4. A diagnostic/prognostic assay according to Claim 3 wherein said assay is an immuno assay.

5. A method for identifying mdm2 interacting proteins employing a yeast 2-hydrid assay comprising;

(i) transforming a suitable yeast strain with a plasmid encoding a binding domain of a transcriptional activator fused in-frame with mdm2;

(ii) growing said transformants under selection;

(iii) re-transforming said transformed yeast strain with a plasmid encoding transactivitation domain, corresponding to said binding domain, fused in frame with a cDNA library;

(iv) growing co-transformants; and

(v) detecting mdm2 interacting proteins by observing transcription and/or translation products;

6. An interaction protein identified by the method according to Claim 5.

7. A method for treating aberrant cell division wherein said method comprises administering to an individual to be treated an effective amount of at least one agent listed in Table 1 or 2, or a homologue or analogue thereof, or a functionally effective fragment thereof, so as to arrest, mitigate or reverse said aberrant cell division.

8. A method for treating HTLV-1 and/or HIV infection comprising administering the therapeutic composition according to Claim 2 to an individual.

9. A therapeutic composition comprising at least one agent represented in Table 1 or 2, or a homologue or analogue thereof, or an effective fragment thereof, excluding S3, S7, L6, EF1a and EF1A for use in the inhibition of translation.

10. Antisense oligonucleotide adapted to hybridize to at least one nucleic acid sequence presented in Tables 1 or 2.

11. Antisense oligonucleotide according to Claim 10 wherein said oligonucleotide is modified as hereindescribed.

12. A pharmaceutical composition comprising the antisense oligonucleotide of Claim 10 or 11.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/02318

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/17 A61K38/41 A61K38/44 C12Q1/02 C07K14/47
C12N15/11 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 420 263 A (BURRELL MARILEE ET AL) 30 May 1995 see example 7 ---	5,6
X	WO 96 12017 A (OTSUKA PHARMA CO LTD ;UNIV AUSTRALIAN (AU); SHINDO YUTAKA (JP); NI) 25 April 1996 see the whole document -& EP 0 781 844 A ---	2-4, 10-12
X	WO 96 23225 A (COR THERAPEUTICS INC) 1 August 1996 see claims 24-26 ---	10-12
X	GB 990 082 A (VOGEL D.A.) 22 April 1965 see the whole document --- -/--	2,9

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
9 January 1998	26. 02. 98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fernandez y Branas,F

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 26973 A (APPLIED GENETICS INC ;HERRNSTADT CORINNA (US); PARKER WILLIAM DAVI) 12 October 1995 see page 46, line 19 - page 49, line 8 ---	10-12
T	WO 97 09343 A (RHONE POULENC RORER SA ;INST NAT SANTE RECH MED (FR); TOCQUE BRUNO) 13 March 1997 see the whole document ---	1-12
A	WO 96 02642 A (UNIV DUNDEE ;PICKSLEY STEVEN MICHAEL (GB); LANE DAVID PHILIP (GB)) 1 February 1996 see the whole document ---	1-12
A	SHEN, R. ET AL: "Identification of the human prostatic carcinoma oncogene PTI-1 by rapid expression cloning and differential RNA display" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 92, 1995, WASHINGTON US, pages 6778-6782, XP002051620 see the whole document ---	1-12
A	WEEDA G. ET AL: "A presumed DNA helicase encoded by ERCC-3 is involved in the human repair disorders xeroderma pigmentosum and cockayne's syndrome" CELL, vol. 62, 1990, NA US, pages 777-791, XP002051621 see the whole document ---	1-12
A	SU, Y.A. ET AL: "Complete sequence analysis of a gene (OS-9) ubiquitously expressed in human tissues and amplified in sarcomas" MOLECULAR CARCINOGENESIS, vol. 15, April 1996, pages 270-275, XP002051622 see the whole document -----	1-12

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 97/02318

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claims 1, 7-8 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 97/02318

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